REMARKS

The Examiner has rejected claims 1-7 as obvious over Watson (Biotechniques, 1997) and Ellidge (US5851808), in view of Stahl (Biotechniques, 1993). Applicants respectfully disagree.

Combined Art Does not Teach "Simultaneously Removing and Circularizing"

Watson teaches use of a modified nucleotide and a repair enzyme to generate a 3' overhang for use in cloning strategies. Watson requires use of unique primer combinations to ensure appropriate assembly because the PCR products are joined in an ordered fashion. Watson could not, as the examiner asserts, "optionally repeat" the steps "until a last PCR fragment is added to the growing chain to produce an assembled fragment" because the PCR fragments are in solution and are not attached to a solid support. Watson does **not** teach use of a site specific recombinase, **nor** use with a solid support, **nor** "simultaneously removing and circularizing."

Elledge teaches use of a *Cre/lox* system to combine two vectors each having a recombinase site and **one of which must have a conditional origin of replication**. Elledge does **not** teach cloning strategies for assembling circularized DNA *de novo* from assembled PCR fragments (linear DNA), **nor** use on a solid support, **nor** "simultaneously removing and circularizing."

Stahl teaches solid phase assembly of oligonucleotides wherein the assembled oligonucleotides are **first eluted** from the solid support, and **then circularized** with a vector and ligase. Stahl does **not** teach use of a site specific recombinase **nor** "simultaneously removing and circularizing."

None of the cited references teach "simultaneously removing and circularizing" as recited in element g) of claim 1. Examiner's arguments fail to establish this element, instead trying to cobble "simultaneous recombination and circularization" (p. 4, OA) plus "removing" (p. 5, OA) from a solid support to somehow equate to "simultaneously removing and circularizing." This is not sufficient to establish a *prima facie* case of obviousness, and withdrawal of the rejection is respectfully requested.

Combined Art Teaches a Different Invention

Finally, if one combines the cited art, what is actually obtained is not the invention, but rather:

> a) Use of a modified nucleotide and a repair enzyme to generate a 3' overhang for use in cloning strategies,
>
> b) together with use of the *Cre/lox* system to combine two vectors each having a recombinase site and **one of which must have a conditional origin of replication,** and
>
> c) solid phase assembly where the DNA is **first eluted** from the solid support, and **then circularized** with a vector and ligase.

Examiner has articulated no rationale for selecting the desired elements and leaving behind the unnecessary elements. The present invention does not require a conditional origin of replication, required for recombination of two plasmids to generate a single plasmid as required by Ellidge. Additionally, the present invention does not require elution and then circularization as required by Stahl.

Because the Examiner has provided **no documentary evidence** supporting the assertion that "simultaneously removing and circularizing" is present in the cited art, and to the extent that Examiner is relying on personal knowledge, inherency or common knowledge in the art to support the rejection, Examiner is respectfully requested to fully articulate the rationale in proper evidentiary form according to MPEP 2144.03,[1] so that Applicants may properly rebut same.

Non-Obvious: First Use of Recombinase on Solid Support

It is not obvious that "simultaneous release and circularization" would be effective using recombinase enzymes on linear DNA tethered to a solid support. The Declaration of Dr. George N. Bennett, submitted herewith, identifies at least three reasons one of ordinary skill in the art would not have thought recombination on a solid support predictable or obvious.

First, the method of releasing a DNA molecule from solid support affects the ability of the DNA to participate in later enzyme reactions. DNA may be denatured, enzymatically altered or degraded when it is released from the solid support. Because **linear DNA had never been**

[1] MPEP 2144.03 ("If the examiner is relying on personal knowledge to support the finding of what is known in the art, the **examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support the finding**. See 37 CFR 1.104(d) (2).") (emphasis added).

released from a solid support by a recombinase, it was unclear how effective this DNA would be for further processing by the recombinase reaction.

Second, the topological structure of linear DNA on a solid support is altered compared to native DNA structures. It is unclear that linear DNA attached to a solid support would be in conformation amenable to recombinase activity. It is also unclear if during recombination, the reaction would be inhibited by conformations induced by the recombinase. The activity of a recombinase on a DNA molecule is affected by the structure and length of the DNA molecule, and generally these difficulties are greater if the DNA molecule is long. Thus assembled linear DNA on a solid support could be **adversely affected** by the structure and size of the DNA in the recombinase reaction.

Finally, recombinase activity is affected by reaction conditions. It was unclear prior to these experiments which conditions (enzyme concentrations and buffers) would allow effective recombination and obtain useful quantities of DNA product especially since tensions on the complex could affect the *Cre/lox* complex activity and ability to complete the reaction. The ability and efficiency of *Cre/lox* recombination for "simultaneously removing and circularizing" linear DNA in **a single step** from a solid support was **unpredictable**. Thus it is not obvious that the Cre enzyme would efficiently remove and circularize immobilized linear DNA substrate from a solid support.

Examiner's Rebuttal Misdirected

The Examiner states that applicants argument "is read to assert the step of site specific recombination and circularization occurring in a single step, with recombinase is inherent in the claim," (p. 5, OA) and thus inherent in the references. However, this rebuttal is misdirected for two reasons. First, Applicant did **not** argue "recombination and circularization occurring in a single step," as suggested by Examiner, instead applicant argued "simultaneous removal and circularization," of an assembled DNA from a solid support. Second, Applicants note, as described above, the difference between the claim and the cited references. In the claim, simultaneous removal and circularization occur because the DNA was on a solid support. This **does NOT happen in the prior art** because the DNA is not on a solid support. Thus, although

"simultaneous removal and circularization" is inherent within the overall claim structure, it is certainly not inherent to the cited references.

CONCLUSION

The art fails to show "simultaneously removing and circularizing" assembled linear PCR fragments from a solid support with a recombinase reaction. Further even if the individual steps were contemplated together it would be highly unpredictable whether the recombinase reaction would perform the steps in a fashion that would allow the formation of an active circularized product in sufficient quantity to be useful. This is the **first demonstration** of recombinase activity on a solid support to produce a circular DNA. In one example, a functional plasmid was assembled *de novo* that could be transformed directly into a cell. As explained above, it was not obvious to use a recombinase with linear DNA tethered to a solid support and Applicants respectfully request the Examiner pass the pending claims to allowance.

The Applicants respectfully request the Examiner contact them if there are any questions or procedures that need to be addressed. No fees are believed to be due for this amendment. However, should there be any additional fees required, please charge such additional fees to Deposit Account No. 50-3420 (reference 31175413-002002 MDB).

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Respectfully submitted,

BAKER & MCKENZIE LLP

By /Michael D. Berger/

Michael D. Berger Ph.D., Patent Agent
Registration No.: 52,616
Pennzoil Place, South Tower
711 Louisiana, Suite 3400
Houston, Texas 77002-2746 USA
Tel: +1 713 427 5031
Fax: +1 713 427 5099
Attorneys For Applicants